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(57) Abstract

A pharmaceutical composition for administration as an injection or as a retention enema contains an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin, with approximately a 1:2 mol/mol stoichiometry the composition including a co-solvent where necessary. The pharmaceutical composition is prepared by dissolving in water an amount of 2-hydroxypropyl-beta-cyclodextrin and then adding, with mixing, an amount of propofol to provide the desired molar ratio, and if necessary, adding the pharmaceutically acceptable co-solvent.

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PHARMACEUTICAL COMPOSITION

BACKGROUND OF THE INVENTION

This invention relates to methods of preparing a pharmaceutical composition for administration as an injection or as a retention enema comprising an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin. Compositions according to the invention are stable non-colloidal aqueous solutions containing up to 30 mg/ml propofol convenient and suitable for direct intravenous administration, or use as a retention enema.

Propofol (2,6 di-isopropylphenol) is widely used as an induction agent for anaesthesia. The high lipophilicity of the agent facilitates rapid penetration of the drug into the central nervous system thus providing rapid onset of action. Owing to the strong lipophilic nature of propofol, the commercial

intravenous preparation is formulated as a lipid emulsion in soya bean oil, egg phospholipids and glycerol. Formation of the emulsion demands sophisticated industrial processes to ensure nanoparticulate dimensions of the lipid phase to enable sterilization by filtration and maintain stability.

Significant pain on injection has been associated with the emulsion formulation. [Propofol , the newest induction agent of anaesthesia; Kanto, J. H. Int. J. Clin. Pharmacol. Ther. Toxicol. 1988, 26 (1) 41-57]. Propofol is currently available commercially as 20 milliliter ampoules containing 10 mg/ml propofol. Propofol is given by intravenous injection for the induction of anaesthesia by administering 40 mg every 10 seconds. Most adults will be anaesthetized by a dose of 2,0 to 2,5 mg per kg body-weight [Martindale 29, 1125]. The same propofol formulation has also been used as a retention enema for pre-operative paediatric sedation, where the contents of the ampoule are introduced rectally. The commercially available emulsion does not however have acceptable retention characteristics.

There is a need for safe and effective intravenous formulations of propofol which are industrially simple to produce and which may cause less pain on injection, and which may also be used as retention enemas.

Cyclodextrins are cyclic oligosaccharides with a cone-like shape. The interior of the cone behaves as ā hydrophobic cavity whilst the exterior of the cone is hydrophilic. The former property enables cyclodextrins to form inclusion complexes with a wide variety of lipophilic molecules which "fit" into the cavity while the latter property facilitates aqueous solubility. Cyclodextrin derivatives such as 2-hydroxypropylated beta-cyclodextrins have been extensively studied for use as parenteral drug carriers owing to their high water solubility and low toxicity [Cyclodextrins in Pharmacy. Frömming, K-

H. & Szejtli, J. 1994 Kluwer Academic Publishers pp 1-44]. A particularly useful grade of 2-hydroxypropyl-beta-cyclodextrin is produced when the degree of 2-hydroxypropylation is controlled to between 3,9 and 5,1 2-hydroxypropyl substituents per beta-cyclodextrin molecule [ZA Pat. No. 84/10042 to Janssen Pharmaceutica] commercially available as Encapsin-HPB™. Clinical studies on 8 healthy volunteers showed that intravenous infusion of Encapsin-HPB at a dose of 3 g, given as a single dose, was safe and well tolerated [Junge, W. and Seiler, K.-U. Janssen Clinical Research Report July 1988].

The following prior art is known in relation to cyclodextrins and propofol.

PCT International patent application WO 9317711 to Australian Commercial Research and Development Limited, Australia teaches the preparation of 6A-amino-6A-N-(4-aminobutyl)-6A-deoxy-beta-cyclodextrin derivatives and formulation of inclusion complexes of propofol or alfaxolone with the modified cyclodextrins.

The preparation and central action of propofol/hydroxypropyl-beta-cyclodextrin complexes in rabbits has been reported [Viernstein, H., Stumpf Ch., Spiegl, P. and Reiter, S. Arzneim. -Forsch. 1993, 43, 818-823]. Solid inclusion complexes of propofol/2-hydroxypropyl-beta-cyclodextrin were prepared by dissolving the propofol in ethanol to obtain concentrations between 10 and 20 %. One part of the drug solution was added to up to three parts of 2-hydroxypropyl-beta-cyclodextrin and kneaded in a mortar until the solvent evaporated. The resulting powder was dried under vacuum to constant weight. Proof of inclusion complexation in the solid state was provided by infrared spectroscopic analysis and differential scanning calorimetry. From phase solubility studies in phosphate buffer the stability

constant of the propofol/2-hydroxypropyl-beta-cyclodextrin complex with 1:1 mol/mol stoichiometry was estimated to be 2940 M^{-1}. The solid complexes were dissolved in artificial plasma, leading to a colloidal solution which was suitable for intravenous administration. Compared with commercial propofol injection no differences were observed between the two dosage forms in the onset, duration and maximal effect.

In a study to determine smooth muscle relaxant effects of propofol and ketamine in isolated guinea pig trachea, propofol was prepared as a 0,1M aqueous solution in 40% w/v 2-hydroxypropyl-beta-cyclodextrin (corresponding to 17,83mg/ml propofol in 400mg/ml 2-hydroxypropyl-beta-cyclodextrin or a mass ratio of 1:22,4 or a molar ratio of 1:2,8 mol/mol propofol to 2-hydroxypropyl-beta-cyclodextrin) and as the commercial lipid emulsion [Pedersen, C.M., Thirstup, S. and Nielsen-Kudsk, J.E. Eur. J. Pharmacol. 1993, 238, 75-80]. Propofol showed three times higher muscle relaxant activity when solubilized with 2-hydroxypropyl-beta-cyclodextrin compared with the lipid emulsion.

SUMMARY OF THE INVENTION

According to a first aspect of the invention there is provided a method of preparing a pharmaceutical composition for administration as an injection or as a retention enema comprising an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with approximately a 1:2 mol/mol stoichiometry, which method includes the steps of:

(a) dissolving in water an amount of 2-hydroxypropyl-beta-cyclodextrin and then adding, with mixing, an amount of propofol to provide an approximate molar ratio of propofol to 2-hydroxypropyl-beta-

cyclodextrin of 1:2 to 1:2,5, to produce a clear colourless solution; and

(b) if necessary adjusting the osmolality of the solution by adding a pharmaceutically acceptable osmolality adjustment agent.

The solution contains the inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with approximately a 1:2 mol/mol stoichometry and is suitable for use as an injection or as a retention enema, to induce sedation or anaesthesia. The excess of 2-hydroxypropyl-beta-cyclodextrin present in the solution acts to stabilize the complex.

According to a second aspect of the invention there is provided a method of preparing a pharmaceutical composition for administration as an injection or as a retention enema comprising an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with a stoichiometry of approximately 1:2 mol/mol, which method includes the steps of:

(1) dissolving in water an amount of 2-hydroxypropyl-beta-cyclodextrin and then adding, with mixing, an amount of propofol to provide an approximate molar ratio of propofol to 2-hydroxypropyl-beta-cyclodextrin of between 1:1,5 and 1:<2, and then adding a pharmaceutically acceptable co-solvent, to produce a clear colourless solution.

Again, the solution contains the inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with approximately a 1:2 mol/mol stoichiometry and is suitable for use as an injection or as a retention enema, to induce sedation or anaesthesia. The excess of propofol present which is not complexed is solubilized by the co-solvent, to give a clear colourless solution.

According to a third aspect of the invention there is provided a pharmaceutical composition produced by either of the two methods described above.

The two methods of the invention may include the additional steps of:

(i) addition to the solution of a pharmaceutically acceptable anti-oxidant such as acetylcysteine and/or EDTA, or sodium metabisulphite, or potassium nitrate and/or a preservative such as benzalkonium chloride or bronopol or chlorhexidine gluconate or chlorobutanol;

(ii) degassing the solution with nitrogen;

(iii) sterilising the solution by filtration; and

(iv) filling the solution into an ampoule.

In the first method of the invention the molar ratio of propofol to 2-hydroxypropyl-beta-cyclodextrin used is approximately 1:2 to 1:2,5 mol/mol, such that the bolus dose of a composition containing 200 mg propofol contains about 3,1 to 4,3g 2-hydroxypropyl-beta-cyclodextrin.

In the second method of the invention there is used a pharmaceutically acceptable co-solvent so that the molar ratio of propofol to 2-hydroxypropyl-beta-cyclodextrin may be reduced to between 1:1,5 and 1:<2 such that the bolus dose of a composition containing 200 mg propofol may contain below 3,0g 2-hydroxypropyl-beta-cyclodextrin.

In step (a) of the first method and step (1) of the second method, the temperature at which dissolution takes place may be between ambient temperature and an elevated temperature up to about 50°C.

In step (a) of the first method and in step (1) of the second method of the

invention, the propofol is preferably introduced in a controlled manner over a period of time with vigorous mixing, to the solution containing the calculated amount of 2-hydroxypropyl-beta-cyclodextrin.

The average degree of substitution of the 2-hydroxypropyl-beta-cyclodextrin used is preferably between 2,5 and 9,0, and more preferably between 3,9 and 5,1 2-hydroxypropyl groups per beta-cyclodextrin molecule.

In step (b) of the first method of the invention, the pharmaceutically acceptable osmolality adjustment agent may be selected from glycerol, dextrose, mannitol and sorbitol. In this step, there may also be a final volume adjustment.

In step (1) of the second method of the invention, the pharmaceutically acceptable co-solvent may be selected from glycerol, a glycol such as propylene glycol or preferably polyethylene glycol with an average molecular mass of 300, a macrogol, or a water soluble polymeric organic compound such as polyvinyl pyrrolidone or a hydroxyalkylated starch derivative such as hydroxyethylated starch with an average molecular mass of 200 000.

The pharmaceutical composition of the invention preferably has a concentration of propofol of 5 mg per millilitre or more preferably about 10 or 20 mg per millilitre.

The composition may be formulated in unit dose form, each unit dose containing from 50 to 400 mg inclusive of propofol. The preferred unit dose contains 200 mg propofol.

The unit dose may be further diluted if required with suitable diluents such as water for injection or dextrose.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the structures and proton notations for propofol and 2-hydroxypropyl-beta-cyclodextrin. The propofol structure (top) shows equivalent protons with prime notation. The 2-hydroxypropyl-beta-cyclodextrin structure (bottom) is composed of seven glucopyranose units shown with the 2-hydroxypropyl ($CH_3CH(OH)CH_2$-) groups omitted which may be substituted on the 2', 3' or 6' hydroxyl oxygens;

Figure 2 shows the assigned 500 MHz proton magnetic resonance spectrum of a solution containing propofol/2-hydroxypropyl-beta-cyclodextrin in a 1:2 mol/mol stoichiometry in deuterated water recorded on a Bruker AMX-500 nuclear magnetic resonance spectrometer;

Figure 3 shows the assigned two-dimensional nuclear Overhauser enhancement spectrum of the propofol/2-hydroxypropyl-beta-cyclodextrin 1:2 mol/mol complex in deuterated water recorded in the rotating frame on a Bruker AMX-500 nuclear magnetic resonance spectrometer;

Figure 4a shows a proton magnetic resonance based molecular model of the aqueous inclusion complex formed between propofol and 2-hydroxypropyl-beta-cyclodextrin, with a perspective view being shown and with the propofol being shown in bold and the 2-hydroxypropyl groups omitted;

Figure 4b shows a space filling model of the complex obtained in Figure 4a

indicating the complete molecular encapsulation of propofol by two cyclodextrin molecules; and

Figure 4c shows the model obtained in Figure 4b with the Z-axis being partially cut away to reveal the interaction between the isopropyl groups of propofol and the hydrophobic cyclodextrin cavity according to the NMR results shown in Figure 3.

DESCRIPTION OF EMBODIMENTS

This invention relates to two methods of preparing a pharmaceutical composition for administration as an injection or as a retention enema comprising an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin. Compositions according to the invention are stable non-colloidal aqueous solutions containing up to 30 mg/ml propofol convenient and suitable for direct intravenous administration. The first method of the invention is preferred.

The major step of both methods of the invention is gradually to add the propofol to a concentrated solution of a calculated amount of 2-hydroxypropyl-beta-cyclodextrin in purified water with vigourous mixing at a temperature from ambient temperature but preferably between 30 and 50 °C. The solution is allowed to cool to room temperature with continued stirring.

In the first method of the invention when no co-solvent is used, the propofol and the 2-hydroxypropyl-beta-cyclodextrin are used in an approximate molar ratio of 1:2 to 1:2,5 to produce a complex with approximately a 1:2 mol/mol

stoichiometry.

In the second method of the invention when a co-solvent is used, the propofol and the 2-hydroxypropyl-beta-cyclodextrin are used in an approximate molar ratio of 1:1,5 to 1:<2 to again produce a complex with a stoichiometry of approximately 1:2 mol/mol.

Beta-cyclodextrin consists of 7 glucose units in a ring structure, also referred to as cyclohepta-amylose. Each glucose unit contains 3 hydroxyl groups which may be etherified by 2-hydroxypropylation to give 2-hydroxypropyl-beta-cyclodextrin (HPB). HPB is produced by reacting beta-cyclodextrin with propylene oxide under controlled conditions so that the average molar substitution (mole propylene oxide per glucose unit) is between 0,25 and 1. This implies that only between 1,75 and 7 hydroxypropyl groups are introduced per beta-cyclodextrin molecule, out of a theoretically possible 21. The average degree of substitution (D.S.) per beta-cyclodextrin molecule may be calculated from the ^{1}H-NMR integrals corresponding to the anomeric and methyl signals between 4,95-5,3 and 0,9-1,2 ppm respectively from the NMR spectrum of HPB in D_2O. The approximate molecular mass may in turn be calculated from the value of D.S.

(1) Calculation of the degree of substitution (D.S.)

$$D.S. = 7b \div 3a$$

wherein a is the integral of anomeric protons and b is the integral of methyl protons.

(2) Calculation of molecular mass (M.M.)

M.M. = (1135 - D.S.) ÷ (59 x D.S.).

Using this method of calculation, and using 2-hydroxypropyl-beta-cyclodextrin with an average degree of substitution of 4,6, the following mol/mol and mass/mass figures are obtained:

Propofol:HPB mol/mol	Propofol:HPB mass/mass
1:1,5	1:11,79
1:2	1:15,72
1:2,5	1:19,65

The average degree of substitution of the 2-hydroxypropyl-beta-cyclodextrin is preferably between 2,5 and 9,0 and more preferably between 3,9 and 5,1 2-hydroxypropyl groups per beta-cyclodextrin molecule. 2-hydroxypropyl-beta-cyclodextrin with an average degree of substitution of 4,6 has a corresponding average molecular mass of around 1400 grams per mole as determined by nuclear magnetic resonance spectrometry. The mass ratio of propofol to 2-hydroxypropyl-beta-cyclodextrin may be between 1:11,0 to 1:20,0 or more preferably between 1:15,72 to 1:19,65, when the average degree of substitution is 4,6 2-hydroxypropyl groups per cyclodextrin molecule.

In the second method of the invention a pharmaceutically acceptable co-solvent or mixture of co-solvents is added to the solution. The co-solvents may be selected from the group consisting of glycerol, glycols, macrogols or suitable water soluble polymeric organic compounds such as polyvinyl pyrrolidone or hydroxyalkylated starch derivatives. Glycerol may be used in the concentration range of 3 to 10 % m/v (mass volume) of the final solution

volume. The glycol is preferably propylene glycol or more preferably polyethylene glycol with an average molecular mass of 300 used in the concentration range of 1 to 10 % m/v of the final solution volume. The hydroxyalkylated starch is preferably hydroxyethylated starch with an average molecular mass of 200 000.

In the absence of co-solvent it may be desirable to modify or adjust the osmolality of the solution by addition of suitable organic osmolality adjustment agents such as glycerol, dextrose, mannitol or sorbitol.

The solution may contain other physiologically compatible compounds such as an anti-oxidant, for example acetylcysteine and/or EDTA, or sodium metabisulphite or potassium nitrate, and/or a preservative, for example benzalkonium chloride or bronopol or chlorhexidine gluconate or chlorobutanol.

The solution is brought to final volume and is degassed with nitrogen. The solution is sterilized by filtration and aseptically transferred into vials or ampoules optionally under a nitrogen atmosphere.

The composition of the invention preferably has a concentration of propofol of 5 mg per millilitre, more preferably about 10 or 20 mg per milliter up to 30 mg per millilitre.

The composition may be formulated in unit dose form, each unit dose containing from 50 to 400 mg inclusive of propofol. The preferred unit dose contains 200 mg propofol.

The composition produced by the methods of the invention may be used for

the induction and short term maintenance of anaesthesia by intravenous injection.

The composition is suitable for further dilution if required in conventional intravenous diluents such as water for injection or dextrose solution.

The composition is suitable for Y-site administration in suitable solutions such as dextrose solution.

The composition is also suitable for use as a retention enema, particularly for pre-operative paediatric sedation. The aqueous nature of the composition has advantages over the conventional emulsion-based formula, as aqueous formulations are generally better retained than oily formulations.

It is to be noted, that as stated above, the reference to Pedersen, C.M., Thirstup, S. and Nielsen-Kudsk, J.E. Eur. J. Pharmacol. 1993, 238, 75-80, teaches that a 17,83 mg/ml solution of propofol is prepared using 400 mg hydroxypropyl-beta-cyclodextrin per ml of solution. This corresponds to a propofol/hydroxypropyl-beta-cyclodextrin mol/mol stoichiometry of 1:2,8 and a lowest mass/mass ratio, irrespective of the degree of substitution of 1:22,43. It has been found that the stability of propofol-hydroxypropyl-beta-cyclodextrin solutions, in the absence of co-solvents, increases with increasing hydroxypropyl-beta-cyclodextrin concentration. However, the amount of hydroxypropyl-beta-cyclodextrin which may be used intravenously is limited by toxicological considerations. This is of special concern in the case of propofol-hydroxypropyl-beta-cyclodextrin complexes since the product may be given continuously by intravenous infusion for maintenance anaesthesia. It has been found that the optimum hydroxypropyl-beta-cyclodextrin concentration to provide a stable aqueous solution containing

propofol in a clinically useful concentration of 10 mg/ml, which may be infused to maintain anaesthesia for at least one hour, without undue hydroxypropyl-beta-cyclodextrin toxicological hazard, is a maximum of about 215 mg hydroxypropyl-beta-cyclodextrin per ml of solution, corresponding to a propofol/hydroxypropyl-beta-cyclodextrin mol/mol stoichiometry of 1:2,5, when the average degree of substitution of 2-hydroxypropyl-beta-cyclodextrin is 4,6.

The following examples relate to the preparation of inclusion complexes between propofol and 2-hydroxypropyl-beta-cyclodextrin, their characterization and pharmaceutical compositions containing them.

Example 1

To a 5 ml glass beaker 0,316 g 2-hydroxypropyl-beta-cyclodextrin D.S. 4,6 (Janssen Biotech) is added. 2,0 ml of 99,8 % deuterated water (Merck) is added and the solution is stirred with a magnetic stirrer until dissolution is complete. The solution is warmed to 45 °C. Pure propofol (Ethyl Corporation) is drawn up into a syringe and 0,02 g is gradually introduced into the solution with vigourous stirring over 5 minutes. Once all the propofol is added the heat is removed and the solution is stirred until ambient temperature is reached. The solution is filtered through a 0,22 micron filter and 1,0 ml is transferred to a 5 mm nuclear magnetic resonance (NMR) tube. The remainder of the solution is analyzed by high performance liquid chromatography (HPLC) for propofol content. The amount of propofol is found to be 10,0 ± 0,1 mg per millilitre.

The NMR sample is placed in the probe of a Bruker AMX-500 NMR spectrometer operating at 500 MHz with probe temperature at 303 K. A one dimensional proton spectrum of the sample is recorded with 128 scans. The

spectral assignments were made on the basis of reported values for cyclodextrins and substituted phenols and on the basis of chemical shift, signal intensity, multiplicity of signals and proton correlations obtained from the two-dimensional spectra. The assigned proton spectrum is shown in Figure 2 with the proton notations shown in Figure 1.

The nature of the inclusion complex formed between propofol and 2-hydroxypropyl-beta-cyclodextrin is directly demonstrable from the following proton magnetic resonance experiment.

Using a standard pulse sequence for Rotating frame Overhauser Enhancement spectroscopy (ROESY) in the TPPI mode, two dimensional spectra of the sample were acquired with a time domain of 256 using a 150 millisecond spin locking pulse and 112 scans. The ROESY experiment reveals through space proton correlations for those protons which are spatially close (< 4 Angstrom). It is well known that the 3' and 5' protons of beta cyclodextrins are oriented towards the centre of the cavity, whereas the 1', 2', 4' and 6' protons are oriented outside the cavity (see Figure 1). Molecular inclusion may thus be directly demonstrated by distance dependant magnetization transfer between propofol protons and the 3', 5' cyclodextrin protons. The assigned two dimensional ROESY spectrum of the complex reveals through space coupling between the magnetically equivalent isopropyl methyl protons of propofol (D,D') and the 3', 5' protons of 2-hydroxypropyl-beta-cyclodextrin as shown in Figure 3. The spectrum thus shows through space correlations between protons separated by a distance of less than 4 Angstrom providing evidence of inclusion complexation. The intensity of the cross peaks is related to the intermolecular interproton distance and thus the nature of the inclusion interaction may be modeled as shown in Figures 4a-c. The structure shown in figure 4a was obtained by

initial molecular mechanics energy minimisation of the X-ray crystallographic based structure of beta-cyclodextrin and a computer generated structure of propofol in energy optimised confirmation whereby the 2-cyclodextrin molecules were allowed to complex with each isopropyl moiety respectively in a trimolecular energy minimisation routine. The commercial molecular modelling software HyperChem™ was used for the calculations and drawings. Owing to the bulky nature of the isopropyl substituents a 1:1 mol/mol stoichiometry would leave a significant hydrophobic protuberance out of the cavity resulting in lower aqueous solubility compared to the 1:2 mol/mol stoichiometry in which the entire propofol molecule is encapsulated as shown in Figure 4b.

Example 2

In an aseptic processing environment 15,82 g 2-hydroxypropyl-beta-cyclodextrin D.S 4,6 (Janssen Biotech) is weighed and transferred to a sterile graduated mixing vessel. Water for injection (20) ml is added with stirring. Stirring is continued until a clear solution is obtained. The solution is warmed to 45 °C. One gram of propofol (Ethyl Corporation) is drawn up into a syringe. The propofol is gradually introduced over a 5 minute period to the concentrated 2-hydroxypropyl-beta-cyclodextrin solution with vigourous stirring. The heat is removed and stirring is continued. When ambient temperature is attained the solution is gradually brought to volume (100 ml) by the addition of aliquots of water for injection with vigorous stirring. Prior to final volume adjustment glycerol is added with stirring to adjust osmolality to 280 - 320 mOsm/kg. The solution is stirred for 10 minutes and bubbled with sterile nitrogen for 20 minutes. The solution is passed through 0,22 micron filter into presterilized glass ampoules. The ampoules are optionally sealed under nitrogen. Propofol content is verified by HPLC analysis to be 10,0 ± 0,1 mg propofol per ml. The clarity of

solution is determined by transmittance spectrophotometry at a wavelength of 800 nanometres and found to be 99,9 %. The solution is stable for at least one month.

Example 3

In an aseptic processing environment 31,64 g 2-hydroxypropyl-beta-cyclodextrin D.S 4,6 (Janssen Biotech) is weighed and transferred to a graduated mixing vessel. Water for injection (40) ml is added with stirring. Stirring is continued until a clear solution is obtained. The solution is warmed to 45 °C. Two grams of propofol (Ethyl Corporation) is drawn up into a syringe. The propofol is gradually introduced over a 5 minute period to the concentrated 2-hydroxypropyl-beta-cyclodextrin solution with vigourous stirring. The heat is removed and stirring is continued. When ambient temperature is attained the solution is gradually brought to volume (100 ml) by the addition of aliquots of water for injection with vigorous stirring. The solution is stirred for 10 minutes and the osmolality of the solution is measured (380 ± 20 mOsm/kg). The solution is stirred for 10 minutes and bubbled with sterile nitrogen for 20 minutes. The solution is passed through 0,22 micron filter into presterilized glass ampoules. The ampoules are optionally sealed under nitrogen. Propofol content is verified by HPLC analysis to be 20,0 ± 0,2 mg propofol per ml. The clarity of solution is determined by transmittance spectrophotometry at a wavelength of 800 nanometres and found to be 99,9 %. The solution is stable for at least one month.

Example 4

In an aseptic processing environment 14,63 g 2-hydroxypropyl-beta-cyclodextrin D.S 4,6 (Janssen Biotech) is weighed and transferred to a graduated mixing vessel. Water for injection (20) ml is added with stirring.

Stirring is continued until a clear solution is obtained. The solution is warmed to 45 °C. One gram of propofol (Ethyl Corporation) is drawn up into a syringe. The propofol is gradually introduced over a 5 minute period to the concentrated 2-hydroxypropyl-beta-cyclodextrin solution with vigourous stirring. The heat is removed and stirring is continued. When ambient temperature is attained 3g of glycerol is added with stirring and the solution is stirred for 5 minutes. The solution is gradually brought to volume (100 ml) by the addition of aliquots of water for injection with vigorous stirring. The solution is stirred for 10 minutes and bubbled with sterile nitrogen for 20 minutes. The solution is passed through 0,22 micron filter into presterilized glass ampoules. The ampoules are optionally sealed under nitrogen. Propofol content is verified by HPLC analysis to be 10,0 ± 0,1 mg propofol per ml. The clarity of solution is determined by Transmittance spectrophotometry at a wavelength of 800 nanometres and found to be 99,9 %. The solution is stable for at least two weeks.

Example 5

In an aseptic processing environment 14,63 g 2-hydroxypropyl-beta-cyclodextrin D.S 4,6 (Janssen Biotech) is weighed and transferred to a graduated mixing vessel. Water for injection (20) ml is added with stirring. Stirring is continued until a clear solution is obtained. The solution is warmed to 45 °C. One gram of propofol (Ethyl Corporation) is drawn up into a syringe. The propofol is gradually introduced over a 5 minute period to the concentrated 2-hydroxypropyl-beta-cyclodextrin solution with vigourous stirring. The heat is removed and stirring is continued. When ambient temperature is attained 10 g of Polyethylene glycol 300 is added with stirring and the solution is stirred for 5 minutes. The solution is gradually brought to volume (100 ml) by the addition of aliquots of water for injection with vigorous stirring. The solution is stirred for 10 minutes

and bubbled with sterile nitrogen for 20 minutes. The solution is passed through 0,22 micron filter into presterilized glass ampoules. The ampoules are optionally sealed under nitrogen. Propofol content is verified by HPLC analysis to be 10,0 ± 0,1 mg propofol per ml. The clarity of solution is determined by transmittance spectrophotometry at a wavelength of 800 nanometres and found to be 99,9 %. The solution is stable for at least two weeks.

Example 6

In an aseptic processing environment, 1000 ml water for injection is heated to 45°C. 1023g 2-hydroxypropyl-beta-cyclodextrin D.S 4,6 (Janssen Biotech) is weighed and added to the water for injection with stirring. Stirring is continued until a clear solution is obtained. 52,0 grams of propofol (Ethyl Corporation) is drawn up into a syringe. The propofol is gradually introduced over a 5 minute period to the concentrated 2-hydroxypropyl-beta-cyclodextrin solution with vigorous stirring using a homogenizer. The heat is removed and stirring is continued for 30 minutes. When ambient temperature is attained the solution is gradually brought to volume (5000 ml) by the addition of aliquots of water for injection with vigorous stirring. The solution is stirred for 20 minutes. The solution is stirred and bubbled with sterile nitrogen until the oxygen concentration is below 0,5 mg/l. The solution is passed through 0,22 micron filter into presterilized glass ampoules. The ampoules are optionally sealed under nitrogen. Propofol content is verified by HPLC analysis to be 10,0 ± 0,5 mg propofol per ml.

The compositions of Examples 2 to 6 may be used either as compositions for injection or as compositions for use as retention enemas, as described above.

CLAIMS

1 A method of preparing a pharmaceutical composition for administration as an injection or as a retention enema, comprising an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with approximately a 1:2 mol/mol stoichiometry, includes the steps of:

(a) dissolving in water an amount of 2-hydroxypropyl-beta-cyclodextrin and then adding, with mixing, an amount of propofol to provide an approximate molar ratio of propofol to 2-hydroxypropyl-beta-cyclodextrin of 1:2 to 1:2,5, to produce a clear colourless solution; and

(b) if necessary adjusting the osmolality of the solution by adding a pharmaceutically acceptable osmolality adjustment agent.

2 A method according to claim 1 wherein in step (a) the temperature at which dissolution takes place is between ambient temperature and an elevated temperature up to about 50°C.

3 A method according to claim 1 or claim 2 wherein in step (a), the propofol is introduced in a controlled manner over a period of time with vigorous mixing to the solution containing the calculated amount of 2-hydroxypropyl-beta-cyclodextrin.

4 A method according to any one of claims 1 to 3 wherein the average degree of substitution of the 2-hydroxypropyl-beta-cyclodextrin used is between 2,5 and 9,0 2-hydroxypropyl groups per beta-cyclodextrin molecule.

5 A method according to claim 4 wherein the average degree of substitution of the 2-hydroxypropyl-beta-cyclodextrin used is between 3,9 and 5,1 2-hydroxypropyl groups per beta-cyclodextrin molecule.

6 A method according to any one of claims 1 to 5 wherein in step (b) the pharmaceutically acceptable osmolility adjustment agent is selected from the group consisting of glycerol, dextrose, mannitol and sorbitol.

7 A method of preparing a pharmaceutical composition for administration as an injection or as a retention enema, comprising an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with a stoichiometry of approximately 1:2 mol/mol, includes the steps of:

(1) dissolving in water an amount of 2-hydroxypropyl-beta-cyclodextrin and then adding, with mixing, an amount of propofol to provide an approximate molar ratio of propofol to 2-hydroxypropyl-beta-cyclodextrin of between 1:1,5 and 1:<2, and then adding a pharmaceutically acceptable co-solvent, to produce a clear colourless solution.

8 A method according to claim 7 wherein in step (1) the temperature at which dissolution takes place is between ambient temperature and an elevated temperature up to about 50°C.

9 A method according to claim 7 or claim 8 wherein in step (1) the propofol is introduced in a controlled manner over a period of time with vigorous mixing to the solution containing the calculated amount of 2-hydroxypropyl-beta-cyclodextrin.

10 A method according to any one of claims 7 to 9 wherein the average degree of substitution of the 2-hydroxypropyl-beta-cyclodextrin used is between 2,5 and 9,0 2-hydroxypropyl groups per beta-cyclodextrin molecule.

11 A method according to claim 10 wherein the average degree of substitution of the 2-hydroxypropyl-beta-cyclodextrin used is between 3,9 and 5,1 2-hydroxypropyl groups per beta-cyclodextrin molecule.

12 A method according to any one of claims 7 to 11 wherein in step (1) the pharmaceutically acceptable co-solvent is selected from the group consisting of glycerol, a glycol, a macrogol or a water soluble polymeric organic compound.

13 A pharmaceutical composition for administration as an injection or as a retention enema comprising a clear colourless aqueous solution of an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with approximately a 1:2 mol/mol stoichiometry.

14 A pharmaceutical composition for administration as an injection or as a retention enema comprising a clear colourless aqueous solution of an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with approximately a 1:2 mol/mol stoichiometry, which pharmaceutical composition is prepared by:

(i) dissolving in water an amount of 2-hydroxypropyl-beta-cyclodextrin and then adding, with mixing, an amount of propofol to provide an approximate molar ratio of propofol to 2-hydroxypropyl-beta-cyclodextrin of 1:2 to 1:2,5 to produce a clear colourless solution; and

(ii) if necessary adjusting the osmolality of the solution by adding a pharmaceutically acceptable osmolality adjustment agent.

15 A pharmaceutical composition for administration as an injection or as a retention enema comprising a clear colourless aqueous solution of an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with approximately a 1:2 mol/mol a stoichiometry and a pharmaceutically acceptable co-solvent.

16 A pharmaceutical composition for administration as an injection or as a retention enema comprising a clear colourless aqueous solution of an inclusion complex of propofol and 2-hydroxypropyl-beta-cyclodextrin with a stoichiometry of approximately 1:2 mol/mol, and a pharmaceutically acceptable co-solvent, which pharmaceutical composition is prepared by:

(i) dissolving in water an amount of 2-hydroxypropyl-beta-cyclodextrin and then adding, with mixing, an amount of propofol to provide an approximate molar ratio of propofol to 2-hydroxypropyl-beta-cyclodextrin of between 1:1,5 and 1:<2, and then adding the pharmaceutically acceptable co-solvent, to produce the clear colourless solution.

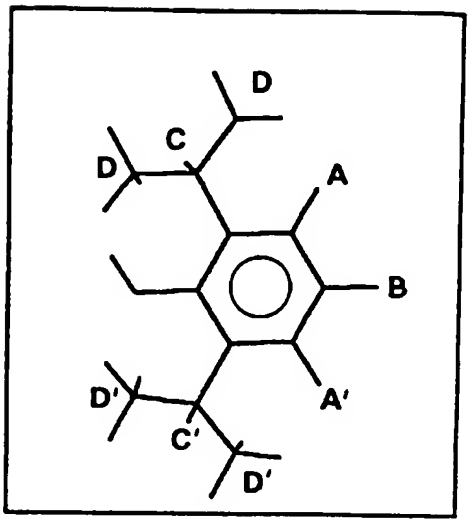
17 A pharmaceutical composition according to claim 15 or claim 16 wherein the pharmaceutically acceptable co-solvent is selected from the group consisting of glycerol, glycol, a macrogol, or a water soluble polymeric organic compound.

18 A pharmaceutical composition according to any one of claims 13 to 17 wherein the composition has a concentration of propofol of at

least 5 mg/millilitre.

19 A pharmaceutical composition according to claim 18 wherein the composition has a concentration of propofol of about 10 to 20 mg/millilitre inclusive.

20 A pharmaceutical composition according to any one of claims 13 to 19 formulated in unit dose form, each unit dose containing from 50 to 400 mg inclusive of propofol.



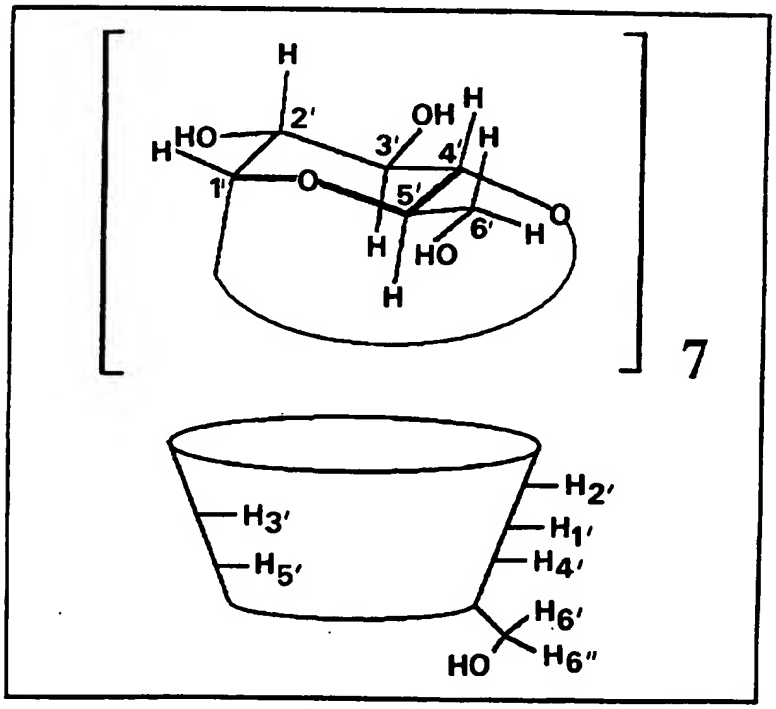
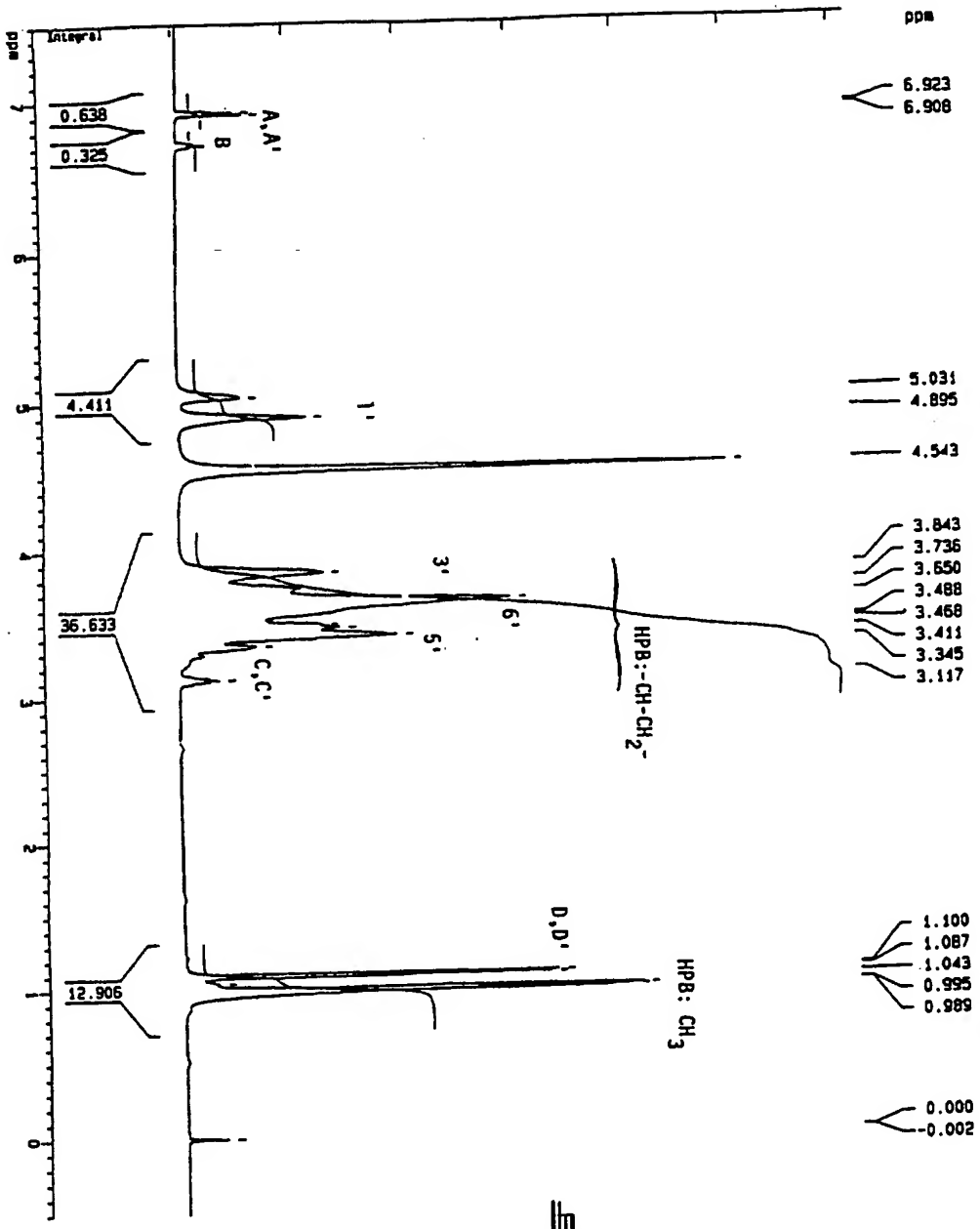


Fig 1


ppm
Integral
0.638
0.325
4.411
36.633
12.906
A,A'
B
1'
3'
6'
5'
C,C'
HPB:-CH-CH2-
D,D'
HPB: CH3
6.923
6.908
5.031
4.895
4.543
3.843
3.736
3.650
3.488
3.468
3.411
3.345
3.117
1.100
1.087
1.043
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0.989
0.000
-0.002

FIG 2

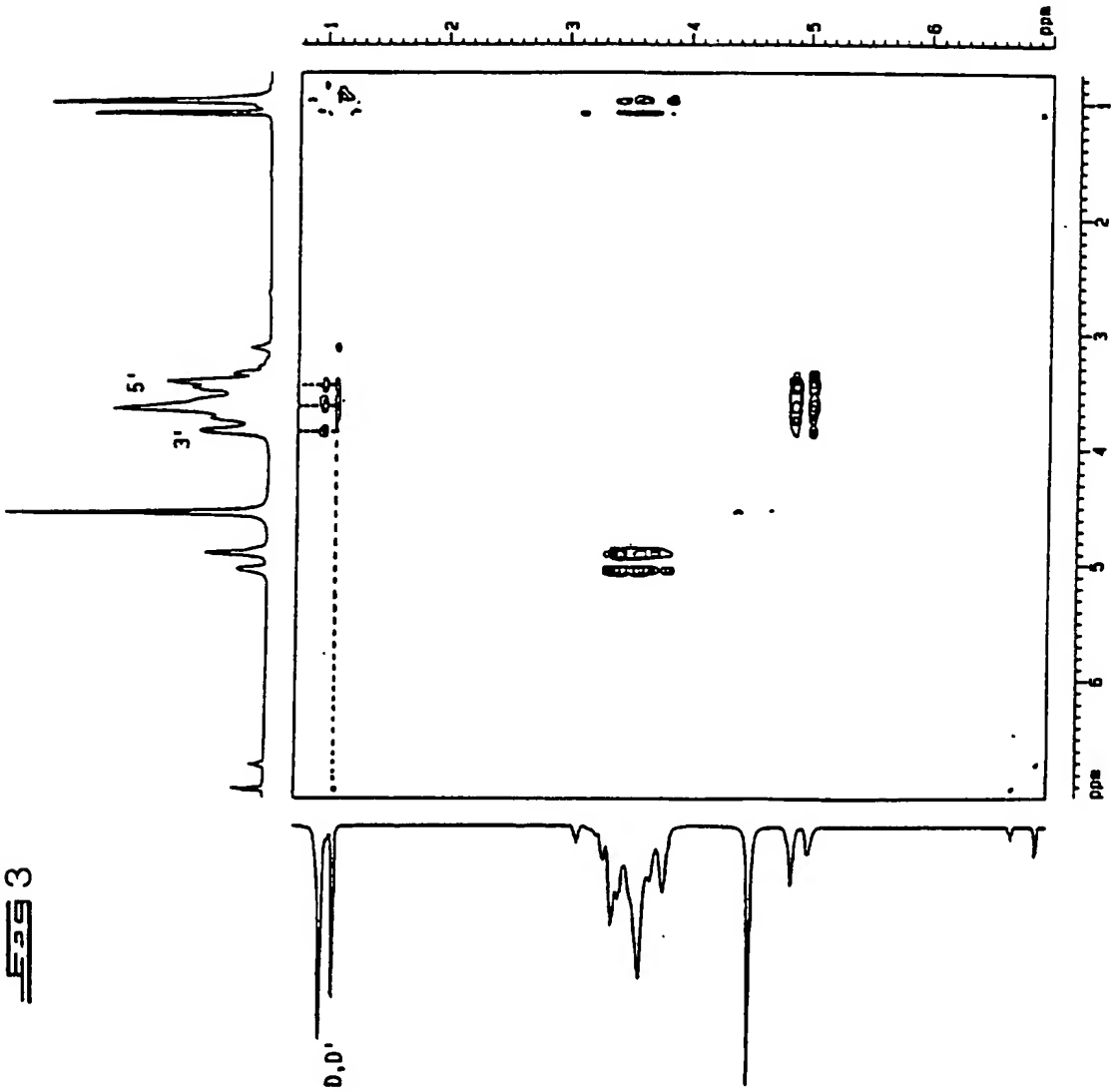

Fig 3

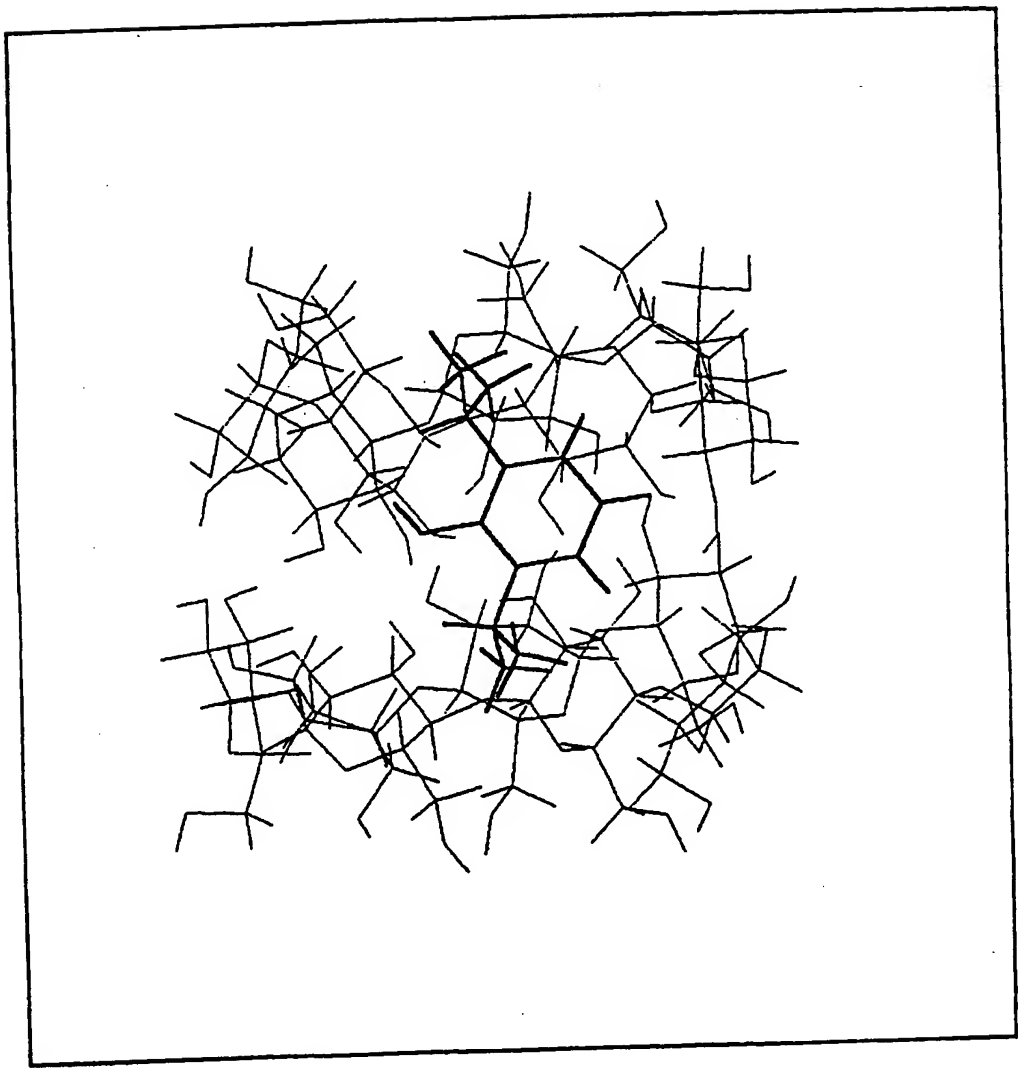

Fig 4a

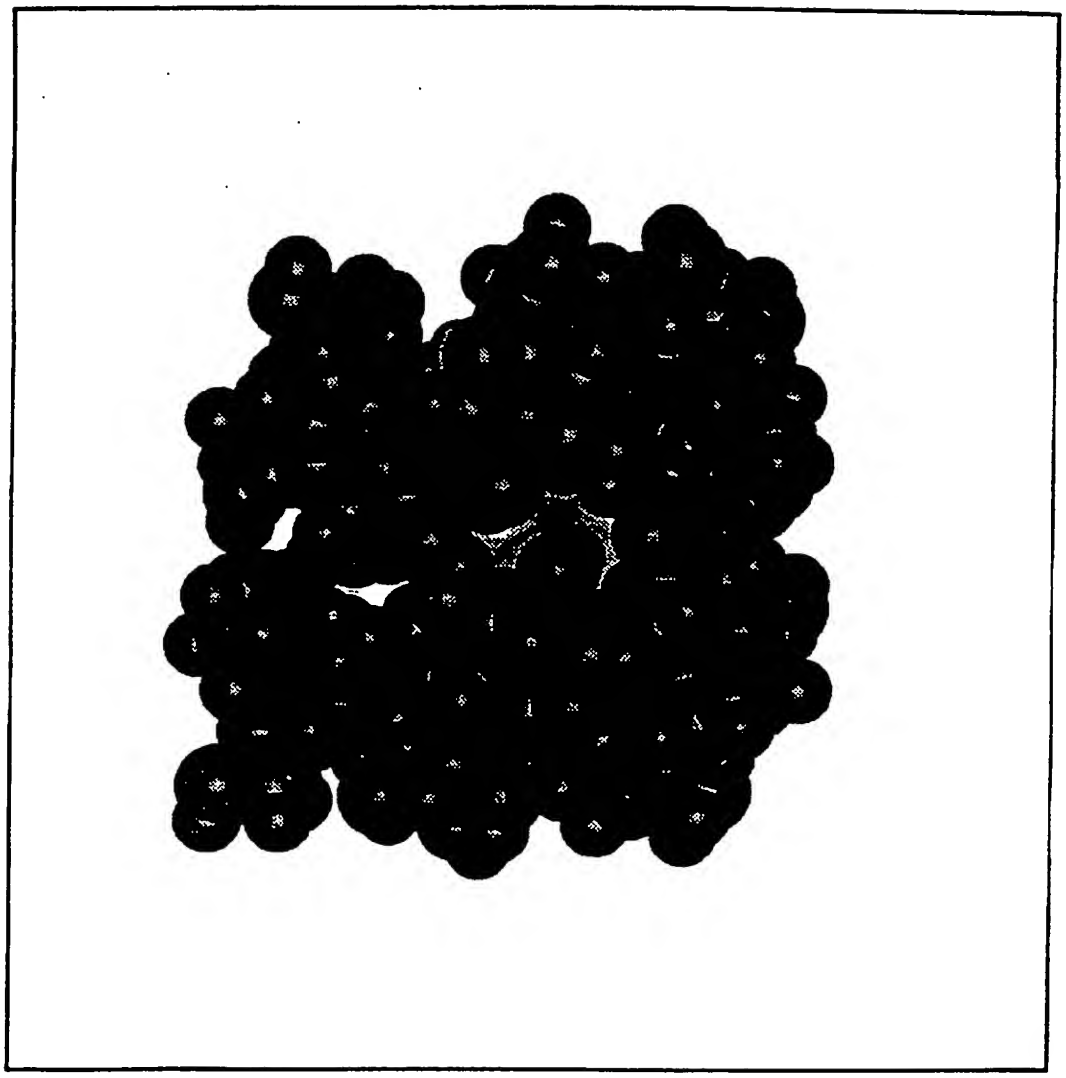

Fig 4b

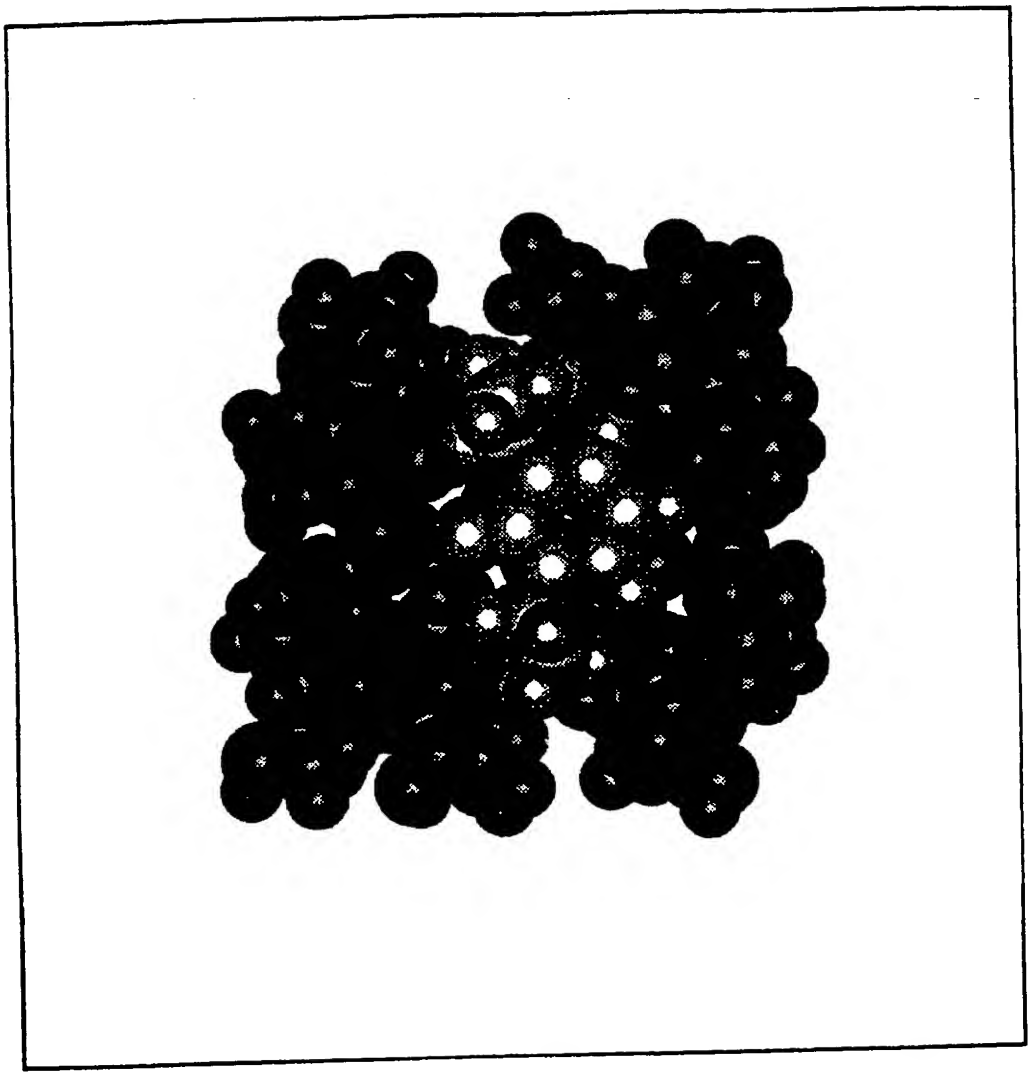

Fig 4c

INTERNATIONAL SEARCH REPORT

Intr ional Application No
PCT/GB 96/00737

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48 A61K31/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 17711 (AUSTRALIAN COMMERICAL RESEARCH) 16 September 1993 cited in the application see page 27, line 2 - line 13; claims	1-20
X	ARZNEIMITTEL FORSCHUNG DRUG RESEARCH., vol. 43, no. 8, 1993, AULENDORF DE, pages 818-821, XP002009536 H. VIERSTEIN ET AL. : "PREPARATION AND CENTRAL ACTION OF PROPOFOL /HYDROXYPROPYL-BETA-CYCLODEXTRIN COMPLEXES IN RABBITS." cited in the application	1
Y	see the whole document	1-20
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
29 July 1996	09. 08. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Berte, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/00737

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 114, no. 10, 11 March 1991 Columbus, Ohio, US; abstract no. 88481, FRIJLINK, H. W. ET AL: "The effects of cyclodextrins on drug absorption. II. In vivo observations" XP002009540 see abstract & INT. J. PHARM. (1990), 64(2-3), 195-205 CODEN: IJPHDE;ISSN: 0378-5173, 1990, ---	1-20
X	EUR. J. PHARMACOL. (1993), 238(1), 75-80 CODEN: EJPHAZ;ISSN: 0014-2999, 1993, XP002009537 PEDERSEN, CHARLES M. ET AL: "Smooth muscle relaxant effects of propofol and ketamine in isolated guinea pig trachea" cited in the application see abstract page 75, ---	1
X	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US AN=97206733, XP002009542 see abstract & 207TH NATIONAL MEETING OF THE AMERIC. CHEM. SOC,, 13 - 17 March 1994, SAN DIEGO, CALIFORNIA, USA, page 207 ---	1
X	PHARM. RES., vol. 8, no. 10, October 1991, page S-157 XP002009538 E. PROP. ET AL.: "BRAIN TARGETORS OF 2,6-DIISOPROPYLPHENOL (PROPOFOL)" see paragraph ---	1-20
P,X	ANESTH. ANALG., vol. 82, 1996, pages 920-924, XP002009539 S. J. BIELEN ET AL. : "THE EFFECT OF A CYCLODEXTRIN VEHICLE ON THE CARDIOVASCULAR PROFILE OF PROPOFOL IN RATS." see page 920, column 2 --- -/--	1,6,13, 20

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INTERNATIONAL SEARCH REPORT

Intr ional Application No
PCT/GB 96/00737

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 123, no. 7, 14 August 1995 Columbus, Ohio, US; abstract no. 83870, BREWSTER, MARCUS E. ET AL: "Solubilization and electrochemical stabilization of substituted phenols through the use of 2 - hydroxypropyl -.beta.- cyclodextrin" XP002009541 see abstract & SUPRAMOL. CHEM. (1994), 4(1), 69-76 CODEN: SCHEER;ISSN: 1061-0278, 1994,	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PC.. GB96/00737

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claims 1-12 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 96/00737

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9317711	16-09-93	EP-A- 0630261	28-12-94